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THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

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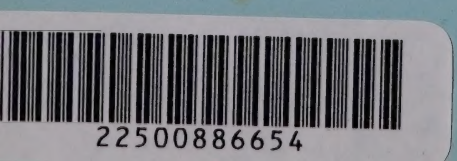

The Formation of the Germ-layers in Crangon vulgaris.

By

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With Plates XX, XXI, and XXII.

The development of the common shrimp has recently been studied by Kingsley,[1] whose papers contain full references to the work of other authors on Crustacean embryology. To these, and to the memoir of Nusbaum [2] on the development of Mysis, the reader is referred for an account of the literature of the subject.

Professor Kingsley's account of the later development of many important organs, especially of those of mesoblastic origin, is somewhat meagre; I hoped, during a recent visit to the Laboratory of the Marine Biological Association at Plymouth, to supplement his work by observations on the later history of the mesoderm. A preliminary study of the mode of formation of the germinal layers has, however, led me to form a conception of the early development of Crangon which differs widely from that arrived at by Kingsley. My observations on the early stages are, therefore, described in what follows.

Embryos of various stages were obtained by the examination of newly-caught females; any attempt to rear the eggs in

[1] 'Bulletin of the Essex Institute,' xviii, p. 99, and xxi, p. 1, Salem, Mass., 1886 and 1889.

[2] 'Archives de Zoologie Expérimentale,' v, 1887.

captivity being rendered useless by the great abundance of material procured for me by the fishermen of the Association. The gravid females were killed by immersion in a solution of corrosive sublimate, warmed to about 50° C., and were afterwards treated with increasingly strong alcohol in the usual way. The shells were easily removed from the eggs with needles; and the eggs themselves were stained, either with picrocarmine in the case of those intended for sections, or when intended for surface views with Grenacher's alum-carmine.

The youngest eggs observed were already divided superficially into four equal segments. The egg at this stage is somewhat elliptical, having a long diameter of 0·5 mm., a short diameter of 0·4 mm. It is surrounded by a single, delicate, transparent shell-membrane, which is, as pointed out by Kingsley, completely filled by the living egg. The appearance of the hardened egg, after removal of the shell, is seen in fig. 1, where the whole surface of each blastomere is seen to be crowded with small spherules of yolk. This spherical condition of the yolk-particles was well retained in all my specimens, though Kingsley appears to have found that the yolk spherules broke up and ran together into masses during the process of hardening. The yolk spheres are so densely crowded on the surface that the nuclei cannot at this stage be rendered visible in the uninjured egg. In sections a single nucleus, surrounded by a small mass of protoplasm, is found in the centre of each blastomere. The protoplasmic mass sends processes into the yolk, which can easily be followed for a certain distance; and there can be little doubt that a protoplasmic reticulum extends between the yolk spherules through the whole substance of each blastomere. Whether a protoplasmic connection between the adjacent blastomeres is present or absent I have been unable to determine.

It is to be noticed that during the stage with four nuclei the segmentation furrows are simple superficial grooves, which do not extend to any considerable distance below the surface of the egg, so that the great mass of the yolk is as yet

undivided. Kingsley, who has described the unsegmented egg and the first two nuclear divisions, states that no furrows appear until four nuclei are present. The rudimentary condition of the furrows in my specimens with four nuclei affords a strong confirmation of this statement.

In the next stage observed eight cells were present, arranged in the manner shown in fig. 2. Round the equator of the egg were four cells arranged in a ring, while at each pole were two cells. The upper and lower cells of fig. 2 are unfortunately so placed that each conceals a second cell. Sections through the eight-celled embryo show that the segmentation furrows are now much deeper than during the four-celled stage. The drawing given in Pl. XX, fig. 10, represents, as accurately as may be, the appearance of a median section. Two of the blastomeres appear to be completely separated, by well-marked fissures, from the rest; but the three remaining cells, through which the section passes, are connected by their central ends with a small, irregular, unsegmented mass of yolk. In other sections of the series a similar communication with the central mass could be demonstrated in the case of every cell.

I have taken some little trouble in order to determine whether this central mass of yolk, with which all the cells appear to communicate, is a mere result of manipulation, or whether it is really present in the living egg. In transparent preparations of the hardened egg, or in living specimens, it seems easy to trace the cell outlines almost to the centre; and examination of specimens in this manner led E. van Beneden to the conclusion that the segmentation was total. But it is difficult to be quite sure of the distinction of the central extremities of cells which are seen through a maze of spherical outlines due to the superficial yolk; and, on the whole, from the great uniformity of the appearances seen in section, it seems probable that the segmentation is really centrolecithal and incomplete, though the central mass of unsegmented yolk is smaller than in many Decapods.

The stage with sixteen cells appears to follow immediately upon that just described, by division of each of the eight blas-

tomeres into two. In this way an equatorial band of eight cells is produced, with a cap of four cells at each pole. An egg at this stage, seen from one pole, is shown in fig. 3. The central yolk-mass is more evident in section than before; and there seems in some cases to be a well-marked difference between the character of the unsegmented mass and that of the yolk contained in the blastomeres themselves. An extreme case of such difference is shown in fig. 11, where the central mass consists of small, highly-refracting spherules, packed closely together, and contrasting strongly with the larger, less refringent masses of the peripheral yolk. This contrast between the two kinds of yolk varies greatly in extent, and may, perhaps, be due to variations in the action of reagents. In the later stages it entirely disappears. As will be noticed in fig. 11, the development of the appearances referred to is frequently accompanied by a very distinct separation between the central yolk-mass and the surrounding blastomeres.

In spite of Kingsley's definite statement to the contrary, I feel sure that the central mass consists, both now and during the whole period of segmentation, of yolk containing only a minimal quantity of protoplasmic reticulum, and entirely devoid of nuclei. Kingsley's statements, which will be discussed below, led me to pay especial attention to the constitution of the central mass; and I have absolutely failed to demonstrate the presence of central nuclei, or of central masses of protoplasm, at any period of segmentation. In sections stained with picrocarmine the contrast is so striking between the brilliant pink of the nuclei, the rosy colour of the protoplasm, and the bright yellow of the yolk, that it would be difficult to overlook a nucleus in the most cursory examination of a series of sections.

The nuclei, as will be seen from fig. 3, do not appear on the surface during the stage with sixteen cells. The stage with thirty-two cells I have not seen; but at sixty-four cells the nuclei are distinctly visible on the surface of the blastomeres (fig. 4).

The regular character of the segmentation is preserved until

128 cells are present, the cell outlines still extending nearly to the centre of the egg. The median section drawn in fig. 12 shows that the central yolk-mass is at this stage composed of elements similar to those of the rest of the yolk. After this period the regularity appears to be lost, for in the next stage I have only been able to count 175 nuclei.

During the later stages of segmentation the nuclei, with their surrounding protoplasm, approach more and more closely to the surface of the egg, while the segmentation furrows become less and less distinct. In the stage with 175 nuclei the furrows can only be traced through about half the distance from the periphery of the egg to the centre (see fig. 13); and at the same time the central yolk exhibits a marked tendency to run together into large, irregular masses under the influence of reagents.

The later stages of the segmentation have not been followed in detail. At the close of this period the egg is surrounded by a layer of cells which are, except in one small region, uniform in character; while the yolk is devoid of nuclei. At one point, which marks the posterior end of the future embryo, is a shallow depression (fig. 5, *Blp.*) lined by cells which are richer in protoplasm than their neighbours, and which are about to divide and to pass again into the yolk.

Professor Kingsley's account of the segmentation differs in many important points from that which is here put forward. According to this observer, the segmentation furrows are throughout confined to the surface of the yolk, so that the greater part of the substance of the egg remains permanently undivided. At the stage with "about sixteen cells," he describes and figures (first paper, p. 106, and fig. 4) a central nucleated mass of protoplasm in the position of the mass described above as unsegmented yolk. He says:

"As will be seen from fig. 4, most of the protoplasm has reached the surface of the egg, but there still remains some near the centre of the yolk. Whether this is the same as the protoplasm described by several authors . . . . I cannot say; but I am certain not only that it is derived from the first seg-

mentation nucleus, but that it plays a part in the formation of the blastoderm."

The later history of this central mass is described as follows:

"While the cells which have reached the surface and which have thus formed a blastoderm are undergoing division, this central protoplasm also divides and migrates, though much more slowly, to the surface," giving rise to the blastoporic patch of cells described above.

This account of the segmentation is so definite, and at the same time so inconsistent with anything which I have myself been able to observe, that I cannot offer any plausible suggestion as to the cause of the differences between Professor Kingsley and myself, unless it be that the mode of segmentation in the same species really differs on the opposite shores of the Atlantic.

The process of formation of the endoderm and mesoderm, which commences at the close of the period of segmentation, proceeds so slowly that these layers are not completely established before the assumption of the Nauplius condition. The stages between the blastosphere already described and the perfect Nauplius will, therefore, be considered together.

The changes in external appearance may be gathered from figs. 5—9. At the close of segmentation the egg is covered, as already stated, by a single layer of cells, which are uniform in character except over a small, depressed area, representing the blastopore. This blastoporic area marks the posterior extremity of the future embryo, and that surface of the egg on which it lies is often slightly flattened, giving an indication of the ventral surface. The relation of the blastoderm to the egg is, however, subject to change, owing to the readiness with which each egg is deformed by pressure.

After the establishment of the blastoporic area, the cells of the ventral surface thicken, and those on each side of the middle line divide, so that an irregular band extends forwards from the blastopore on either side, in which the nuclei are more

crowded than over the remainder of the egg. The distribution of nuclei at this stage is shown in fig. 5*a*.

Shortly after the establishment of two ventral bands of crowded nuclei, each band becomes divided into three distinct regions: an anterior optic lobe, corresponding to the cephalic lobe described by Reichenbach in Astacus;[1] a median region, in which the nuclei are less densely crowded; and a posterior thickened and densely nucleated region, corresponding to the thoracico-abdominal plate of Reichenbach, or to the ventral (neuro-muscular) plates described by Kleinenberg in the larva of Lopadorhynchus.[2] The optic lobes (fig. 6, *o. p.*) are characterised by the more or less definitely concentric arrangement of their closely-packed nuclei. The region between these and the ventral plates will ultimately give rise to the two pairs of antennæ and to the mandibles; while the post-mandibular ectoderm, together with the whole mesoderm of the body, arises from the ventral plates. These ventral neuro-muscular plates (fig. 6, *n. m. p.*) are more conspicuous than the remaining portions of the ventral bands, because their surface is externally concave (compare the section, fig. 16). The nuclei in these plates are arranged in irregularly concentric rings, like those of the optic lobes. The irregularity of these nuclei is perhaps exaggerated in the figure. The blastopore is not closed, as Kingsley erroneously supposes, but may be distinctly recognised as a very small pit, surrounded by a ring of nuclei, between the posterior margins of the ventral plates (see fig. 6, *Blp.*). This embryo, therefore, corresponds fairly well with Reichenbach's stage, as shown in his fig. 3, pl. ii; the only important difference between the two arising from the very small size of the blastoporic patch in Crangon as compared with that of Astacus.

There is a considerable gap between the stage just described and that shown in fig. 7; the changes which occur in the interval will, however, be readily understood from the figures themselves, and from the sections of intermediate stages to be presently described.

[1] 'Studien z. Entw. d. Flusskrebsse,' Frankfurt a/M.

[2] 'Zeitschr. f. w. Zoologie,' Bd. xliv, 1886.

The embryo drawn in fig. 7 corresponds to Reichenbach's stage EF, figured on pl. iii, fig. 8, of his work. The optic lobes have much the same appearance as that already seen in fig. 6, while the bands connecting these with the ventral plates have made considerable progress. The first antennæ are already visible as well-marked hemispherical projections, while a ganglionic rudiment appears as an aggregation of nuclei at the base of each. The second antennæ are indicated, behind the first, by a slight increase in the crowding of the nuclei, which in this place exhibit to a marked degree the curious arrangement in intersecting curves which is so well seen in Reichenbach's figures of Astacus. Behind the second antennæ, and just in front of the ventral plates, the mandibles are already present as a pair of slight projections. The concavity of the ventral plates is well marked at this stage, and causes these structures to appear separated from one another in the middle line by a raised ventral crest. A quite similar horizontal ridge seems to bound the ventral plates anteriorly; and this ridge has apparently been mistaken by Kingsley for the commencement of a pit, which will ultimately, according to him, produce the ventral flexure of the body characteristic of the later stages. Kingsley appears to have examined a section passing through the blastoporic pit (which he calls "proctodeum") and through the concavity of one ventral plate, which he has regarded as representing the commencement of an abdominal flexure; but his figures are very difficult to reconcile with the appearances seen in sections by myself.

Between the first antennæ is a median aggregation of nuclei; and behind this, on a level with the posterior border of the antennules, is a small depression, which is distinctly visible in sections, but which could not be satisfactorily shown in fig. 7. This depression is the mouth; and from its position in this and in the next stage (compare fig. 8) the first antennæ are evidently præoral from the very earliest period at which the mouth is visible. This view of the relations between the mouth and the first antennæ is, I think, unquestionably justified by the figures referred to, together with those of the

sections represented in figs. 23 and 24; but it is in flat contradiction to Kingsley's statements, and to his remarkable fig. 32 (second paper, pl. i), in which a black dot placed between the optic lobes is called the mouth.

It is to be observed that during the stages of figs. 6 and 7, the ventral bands which form the blastodermic area occupy nearly half the surface of the egg. During the following stages a remarkable shrinking occurs; so that the Nauplius occupies a portion of the surface of the egg smaller than that occupied by the newly-established germinal bands. Kingsley has called attention to this shrinking of the embryonic area in Crangon; and a similar shrinking was observed in Pagurus by Paul Mayer.

At the stage represented in fig. 8, all the Nauplius appendages have already become distinct. The optic plate has the form of a rounded lobe, distinctly marked off from the rest of the blastoderm behind, but passing gradually into it in front. The first antennæ are larger than the other appendages, and are dilated at their extremities. Their præoral position is at this time unmistakable, the mouth appearing as a narrow transversely elongated slit, bounded by closely-set nuclei, at the level of the interval between the first and second pairs of appendages. The second antennæ and the mandibles are simple, rounded papillæ. The ventral plates have ceased to be prominent externally, though they are, as will be seen, easily recognised in section. The thoracico-abdominal rudiment has the form of a rounded papilla, projecting slightly from the posterior surface of the blastoderm, but connected with the cephalic region by an even slope. There is at this stage no sharp flexure of the posterior part of the embryo upon the head, such as is seen in the next stage. It need hardly be pointed out, that the existence of such a stage as that here described is quite incompatible with the formation of an abdominal flexure at an earlier period by the appearance of the epiblastic pit described by Kingsley. The nuclei of the whole post-oral region of the body exhibit a marked tendency towards an arrangement in parallel rows, lying

transversely to the long axis of the embryo. In the thoracico-abdominal region this transverse arrangement is particularly well seen; the nuclei in this region forming a series of rings, concentrically arranged round a point which occupies the apex of the papilla, and which indicates the position of the now closed blastopore.

The last stage figured is a fully-formed Nauplius (see fig. 9) which has already undergone an ecdysis. The external appearance of this embryo at this stage is greatly modified by the growth of the thoracico-abdominal papilla, which has greatly increased in size, and has at the same time become folded forwards over the cephalic blastoderm. The optic lobes are larger than before, and each lobe is divided into an outer retinal portion, in which the nuclei are larger, and exhibit a more definitely concentric arrangement; and an inner, ganglionic region, with smaller and more densely crowded nuclei. The optic ganglion is continuous with that at the base of the first antennæ. The first antennæ themselves are much larger than before; the free portion of each projects transversely outwards for nearly half its extent, and then becomes bent at right angles, so as to project directly backwards. The bases of the first two antennæ are connected by a prominent ridge, which is crowded with nuclei, and which overhangs the mouth; so that the mouth itself is not visible in the uninjured Nauplius. The second antennæ arise behind the first, and below (dorsal to) the transverse swelling just described; they are already distinctly biramous, the outer branch being the larger. The mandibles are partly concealed by the antennæ, and are still relatively small; they are seen in section to be distinctly biramous. The thoracico-abdominal rudiment is now much swollen at its base, and is prolonged at its extremity into a flattened papilla of considerable size, with a rounded and very slightly emarginate apex, which is folded over the cephalic portion of the embryo. This mode of origin of the ventral flexure, by the growth of a papilla which bends forwards as it becomes larger, is quite similar to that described by Reichenbach in Astacus, and by Nusbaum in

Mysis. It is, however, quite incompatible with the account given by Kingsley, and already referred to, of the process in Crangon itself. The transverse arrangement of the nuclei is somewhat obscured in surface views by the peculiar curvature of the embryo; but it is, as will be seen, more evident in sections during this stage than in younger embryos.

From the stage represented in fig. 7 to the Nauplius stage a small patch of thickened ectoderm-cells—the "dorsal organ"—is present on the posterior dorsal surface of the embryo. I have nothing to add to the account of this structure which has been given by Kingsley. Its later history, together with that of other organs, I hope to describe in a future paper.

The internal changes which accompany the development may now be described.

Immediately after the formation of the blastoporic area shown in fig. 5, an invagination commences. The cells of the blastoporic area divide, and apparently become amœboid, some of them travelling from the surface of the blastoderm into the substance of the yolk. Certain of these cells, which evidently correspond to the "lower-layer cells" (vitellophags) of Nusbaum, send out processes in all directions among the spherules of the yolk, and become irregularly distributed through its substance. Other cells remain, for a considerable time after their invagination, in the immediate neighbourhood of the blastopore. As will appear directly, I am inclined to regard the whole of the invaginated cells as forming endoderm; and I have failed to find any evidence of an ultimate formation of blood-corpuscles from the cells which first split off from the blastoderm. I have also failed to recognise the mesoderm cells spoken of by Kingsley, who distinguishes "some cells with large nuclei and amœboid outlines, which are plainly budding out from cells at the mouth of the blastopore, and sinking into the yolk." In Kingsley's figure (first paper, fig. 9) there is no apparent evidence of any difference between the various cells which are undergoing invagination; and no explanation is attempted of the manner in which these so-called mesoderm-cells separate from the rest of the un-

doubted endoderm, and migrate towards the ventral surface of the yolk to form the mesodermic bands.

The appearance of a section passing through the blastopore during this process of invagination is shown in fig. 14. The cells of the ectoderm, which cover the greater part of the surface of the embryo, are seen to be even more superficial than at the close of segmentation; and the divisions between them extend only for a very short distance into the yolk. The great mass of the yolk is therefore undivided; and into this undivided mass the ectoderm-cells wander. The figure is drawn with a considerable amount of care, and represents with fair accuracy the appearance of a typical section. It will, I think, be admitted that there is no difference in character between the invaginated cells so great as to enable any observer to say that some are endodermic and others mesodermic in nature.

Immediately after the invagination of the amœboid cells shown in fig. 14, the ventral surface of the blastoderm thickens, as is indicated by the crowding of the nuclei in fig. 5*a*.

In embryos of the age of fig. 6, the endoderm is separated into two distinct portions: one of these is formed by the cells which were first invaginated (vitellophags of Nusbaum), and which have by this time become scattered irregularly through the substance of the yolk (figs. 15 and 16, *En'*); the other forms a more or less compact mass of cells, confined to the posterior portion of the embryo, and continuous with the cells of the persistent blastoporic area (fig. 15, *En''*). The posterior mass of endoderm frequently contains a small lumen, such as that shown in fig. 15; but this lumen is not always demonstrable, and I have never been able to show that it communicates with the exterior.

The relations of the ectoderm at this stage will be understood from figs. 15 and 16, one of which represents a median, the other a lateral longitudinal section. The ectoderm is everywhere one cell deep; in the middle ventral line (fig. 15) the cells are only slightly thicker than those outside the em-

bryonic area; but in the region of the ventral bands the thickening is greater, especially in the optic lobes and in the ventral plates. The optic lobes (fig. 16, *o. p.*) consist of long, closely-packed cells, with elongated nuclei, the cells of the ventral plate being broader in proportion to their length, with more nearly spherical nuclei. The concavity of the ventral plate is fairly well seen in fig. 16.

The condition of an embryo intermediate between figs. 6 and 7 is represented in figs. 17—22. The endoderm is seen to differ from that of younger embryos chiefly in the greater size of the posterior portion, which is still continuous with the blastoporic area. In the specimen from which the transverse sections, figs. 20 and 21, were prepared I could find no trace of a lumen in the posterior endoderm. An egg, taken from the same mother, was cut longitudinally, and the median section (fig. 22) shows a distinct cavity, which does not communicate with the exterior. The lumen is present in about half my series of sections through embryos of this age. It is very difficult to determine whether the increase in the amount of posterior endoderm which has taken place since the last stage is due to a continuation of the process of invagination, or to simple division of the previously invaginated cells. The appearances indicated by figs. 20—22 seem equally consistent with either view. An interesting feature of the posterior endoderm is its apparent tendency to become continuous with the ectoderm in the middle ventral line. The section drawn in fig. 21 is near the anterior limit of the original blastoporic area, and that shown in fig. 20 is the sixth of a fairly thick series of sections in front of it. In the anterior section the large endoderm-cell seemed to be distinctly continuous with the small cells between the ventral plates. This kind of appearance will be noticed in sections through the later stages. It is, of course, possible that these appearances of a fusion between endoderm and ectoderm are merely accidental; but it is equally possible that they are indications of a ventral elongation of the blastopore, in which case they will be regarded by many morphologists as of great importance.

The anterior scattered endoderm-cells have increased in number since the last stage, partly, no doubt, by division of previously scattered cells, but partly by the migration of cells from the posterior endoderm. Such a migration of cells is distinctly indicated in fig. 22. A fair idea of the number and distribution of these scattered cells in the anterior part of the body may be gathered from figs. 18 and 19.

The ectoderm has undergone few changes of importance. The optic lobes (fig. 17) have the same structure as before; behind these, in the region of the first antennæ, the ventral ectoderm is slightly thickened (fig. 18), while still more posteriorly the stomodæum is already visible as a slight pit, hollowed out in a thickened mass of ectoderm. It will be understood that the apparent symmetry of the mouth in fig. 19 is due to the obliquity of the section.

In the region of the trunk the ventral plates are well seen (figs. 20 and 21) as concave thickened plates. Anteriorly these plates are still one cell thick, but behind the cells are in places arranged in two layers. In some sections (one of which I have purposely drawn in fig. 21) the appearances are consistent with the possibility that the cells of the inner layer (the future mesoderm) are added to the ventral plate by migration of cells from the primitive endoderm; but I feel convinced, from the examination of many series of sections, that this is not the case. The elongated cell on the right-hand side of fig. 20, the spindle in an undoubted ectoderm-cell of fig. 27, and the general appearance of the ventral plates in fig. 29 give evidence, of a kind which might have been indefinitely multiplied, that the doubling of the layers in the ventral plates is really due to a division of the ectoderm-cells. The doubtful case shown in fig. 21 has been given principally in order to do the fullest possible justice to Professor Kingsley's contention that the primitive mesoderm is entirely invaginated from the blastopore.

When the stage represented in fig. 7 is fully attained, both ectoderm and endoderm have made important progress. The endoderm, in the region of the ventral plates, is in much the

same condition as before, but the lumen, which was at least occasionally present during the earlier stages, has now entirely disappeared; and the whole posterior portion of the endoderm is now a branched vacuolated mass. The blastoporic area is no longer conspicuous in surface views of the embryo, though its position is indicated by the angle between the posterior portions of the ventral plates (fig. 7, *Blp.*); but the region of continuity between ectoderm and endoderm in the middle ventral line is even more conspicuous than before (fig. 27). The separation between the lateral endoderm and the ventral plates is even more conspicuous than before. The cells of the anterior endoderm have become more abundant; and while certain of their number remain scattered through the yolk, others are beginning to arrange themselves in a layer on the ventral surface of the yolk, so that the alimentary canal begins to acquire a definite epithelial floor. This layer of endoderm is already fairly complete in the region of the mandibles and of the second antennæ, while further forwards it is still imperfect (compare figs. 23—26, *En'*). Posteriorly, the epithelial layer of anterior endoderm becomes continuous with the posterior mass already described. This anterior layer of endodermal epithelium appears to correspond, on the one hand, with the similar layer of anterior endoderm described by Nusbaum in Mysis, and on the other with the layer of flattened cells, fusiform in section, which are stated by Kingsley to represent the cephalic mesoderm.

The ectoderm has become specialised, in the region of each optic lobe, into a retinal plate, which is one cell thick, and a ganglionic region, consisting of several layers of cells. The appearance of the optic lobes in section is nearly the same as that shown in fig. 28, from an older embryo. The first antennæ appear immediately behind the optic lobes (fig. 23, *Ant. i*), and at the inner side of the base of each is an aggregation of ectodermal nuclei representing a commencing ganglion (fig. 23, *N. S.*). Five sections behind the level of fig. 23 appears the stomodæum (fig. 24, *Stom.*), on each side of which is an aggregation of nervous nuclei. The antennules are almost

entirely free from the body in the section figured. The second antennæ are represented simply by densely nucleated portions of ectoderm (fig. 25) which do not as yet project beyond the surface, while the mandibles are already visible as slight projections (fig. 26, *Md.*).

The ventral plates (fig. 27, *n. m. p.*) are thicker than in previous stages, both because the individual cells are larger, and because they are arranged more completely than before in a double layer. The section figured is especially fortunate, because the spindle which appears on the right-hand side shows distinct indication of a transverse division of the ectoderm-cell to which it belongs. The very remarkable appearance of the nuclei of the left ventral plate may, perhaps, be an indication that these also are about to divide.

In the next stage (fig. 8) the principal changes affect the external form. The endoderm is practically unaltered, except for the further addition of wandering anterior cells to the ventral cephalic layer. The ectoderm is pulled out, as already stated, into well-marked processes which form the three pairs of appendages, and the structure of the optic lobes is slightly more complex than before. The division into a retinal plate (fig. 28, *r. p.*) and a ganglionic region (*o. g.*) is sufficiently obvious. The ganglia are confluent in the middle line, and partly overlap the retinal plate. The section figured being somewhat oblique, the retinal plate appears free on one side, while on the other it is partly covered by nervous cells.

The ventral plates (fig. 29, *n. m. p.*) have much the same appearance as that seen in older embryos of the last stage; they are, however, still more distinctly two-layered. Between these plates, in the middle line, the appearance of a fusion between ectoderm and endoderm is still retained.

With the growth of the thoracico-abdominal papilla the endoderm and the ventral plates undergo important changes. A horizontal longitudinal section through the papilla of an embryo intermediate between figs. 7 and 8 is shown in fig. 30. With the growth of the thoracico-abdominal papilla, the posterior endoderm is drawn upwards into the cavity of that

structure; and at the same time the endodermal cells arrange themselves in the form of a tube, closed posteriorly and dorsally, where it is in contact with the former blastoporic area, and widely open anteriorly, where its funnel-shaped mouth embraces the yolk, its ventral wall being continuous with the ventral sheet of cephalic endoderm already described. The ventral plates commence at this time to increase in size; and at the posterior extremity of each a pair of large cells is found (fig. 30, *m. b.* and *e. b.*), one cell being especially related to each of the two layers which form the plate. These cells are evidently homologous with the "Knospungszellen" of Reichenbach, and may be spoken of as ectoblasts and mesoblasts respectively.

I regret that in spite of numerous attempts I have been unable to find an embryo which showed the exact mode of origin of these large cells; but there can, I think, be little doubt that they arise as specialisations of cells which already existed in the ventral plates during previous stages, and not by the addition to these plates of new cells derived from the endoderm.

When the embryo has reached the stage represented in fig. 19, the budding cells have increased in number, and each has a fairly definite relation to a band of cells on the ventral side of the embryo. The flexure of the thoracico-abdominal papilla prevents these cells from appearing in surface views; but their relations will be apparent from the horizontal longitudinal sections of the papilla which are represented in figs. 31—33, and which cut the embryo in planes parallel to the line *x y* in fig. 34, and perpendicular to the plane of the paper. Of these sections, fig. 31 represents the most dorsal, fig. 33 the most ventral. In fig. 31 the tubular portion of the posterior endoderm is well seen; and its perfect continuity with the cells of the first-formed endoderm, which by this time form a fairly regular layer on the ventral surface of the yolk, is also evident.

The section passes through a single mesoblast on each side, which lies at the extremity of a considerable plate of well-

differentiated mesoderm. On one side there is a single ectoblast outside the mesoblast, on the other there are two such cells. In fig. 32, which is ventral to fig. 31 and separated from it by the thickness of two sections, two more mesoblasts are present, and the mesoderm extends forwards from these in the form of a pair of broad bands, meeting in the middle line below the alimentary canal, but not as yet extending into the cephalic region. In several series of sections, cutting embryos of this stage in various planes, I have been able to recognise four large mesoblasts, and four only. I am, therefore, inclined to believe that the number is constant. Outside the mesoblasts in fig. 32 are seen two more ectoblasts; but the definite relation of the ectoblasts to the cells of the ventral and lateral ectoderm can only be fully realised by the examination of superficial sections, parallel to the surface of the ectoderm. Such a section is represented in fig. 33, where four ectoblasts are seen, each of which is at the posterior extremity of a row of ectodermal cells. The nuclei of these ectoderm cells are so arranged that they fall with equal facility into transverse or longitudinal series. The transverse arrangement is most striking in views of the whole embryo, and has already been alluded to; but the reality of the relation to the ectoblasts, indicated in fig. 33, can hardly be doubted. This arrangement of the ectoderm-cells can be traced for a considerable distance along the ventral face of the cephalic region; but those sections in which it is best seen must necessarily cut the embryo in various oblique planes, and explanation of them would be long and tedious; figures of such preparations have, therefore, been omitted.

The relations of mesoderm and endoderm can, perhaps, be more clearly gathered from the lateral longitudinal section shown in fig. 34, in which the connection between the tubular posterior endoderm and the ventral layer produced by the rearrangement of the first-formed anterior endoderm-cells is particularly well seen.

The structure of the remaining organs of the Nauplius may be most conveniently considered in connection with the history

of the later development, and a fuller description of the embryo at this stage is therefore deferred.

The agreement between the account here given of the formation of the layers in Crangon, and that given by Nusbaum of the corresponding process in Mysis, is on the whole fairly complete; but there are, nevertheless, certain points of difference which are not unimportant. The cells which are here spoken of as anterior endoderm correspond closely in their mode of origin with Nusbaum's "Vitellophags," being cells which separate from the rest of the blastoderm before the main body of the endoderm, and which immediately wander through the yolk. The corresponding cells of Mysis differ from those seen by me in the fact that they are separated from the superficial portions of the blastoderm at a slightly earlier stage; but they resemble the cells of Crangon in arising from the blastoporic area, which serves in both cases as a centre of dispersion, from which the cells scatter through the yolk. The statement repeatedly made by Nusbaum that these cells take no part in the formation of the alimentary epithelium does not seem to me to be proved either by his descriptions or by his figures. The extreme rarity of the "Vitellophags" in all those stages in which an endodermic epithelium occurs makes an interpretation of Nusbaum's figures, similar to that which is here offered in the case of Crangon, seem perfectly possible. The formation of a tubular endodermal tract, open in front towards the yolk, and early connected with a ventral anterior sheet of endoderm is a point in which the the two genera closely agree.

The formation of the mesoderm from two ventral ectodermal plates is also described by Nusbaum. In Mysis, however, these plates appear to extend further forwards than in the shrimp, and a formation of a zone of large budding cells does not occur.

The many points of difference between Professor Kingsley and myself have been already alluded to, and I can only express regret for their existence, without being able in any

way to suggest a possible reason for the extremely different results at which we have arrived.

The account here given of the early development suggests many interesting comparisons with corresponding stages of other forms, and especially with Lopadorhynchus, but I prefer to postpone a discussion of these points until I am in a position to describe the later history of Crangon.

EXPLANATION OF PLATES XX, XXI, and XXII,

Illustrating Professor Weldon's paper on "The Formation of the Germ-layers in Crangon vulgaris."

List of Reference Letters.

Ant. i. First antenna. *Ant. ii.* Second antenna. *Blp.* Blastopore. *c. o. p.* circumoral portion of ventral plate. *Eb.* Ectoblast. *En'.* Endoderm which is just invaginated as scattered cells. *En''.* Continuous endoderm of later invagination. *Mb.* Mesoblast. *Md.* Mandible. *me.* Mesoderm. *n. m. p.* Neuro-muscular plate. *N. S.* Nerve-cord. *o. g.* Optic ganglion. *o. p.* optic plate. *r. p.* retinal plate. *Stom.* Stomodæum. *Th. abd.* Forecast of the thoracico-abdominal segments.

Figs. 1—4.—External views of segmentation.

Fig. 5.—Embryo with newly-formed blastopore.

Fig. 5*a*.—Outline of embryo slightly older than Fig. 5, showing the crowding of nuclei in the region of the future ventral bands.

Fig. 6.—Embryo with fully-formed ventral bands and conspicuous blastopore.

Fig. 7.—Embryo with optic plate, first antennæ, and mandibles.

Fig. 8.—Embryo with Nauplius appendages, before the formation of an abdominal flexure.

Fig. 9.—Fully-formed Nauplius, after ecdysis, with well-marked abdominal flexure.

Fig. 10.—Section through an eight-celled egg.

Fig. 11.—Section through an embryo with sixteen cells.

Fig. 12.—Section through an embryo with 128 cells.

Fig. 13.—Portion of a section through the centre of the egg at the close of segmentation.

Fig. 14.—Section passing through the blastopore at the stage shown in Fig. 5.

Fig. 15.—Median longitudinal section through an embryo of the age of Fig. 6.

Fig. 16.—Lateral section from the same series.

Figs. 17—21.—Transverse sections through an embryo slightly older than Fig. 6.

Fig. 22.—Posterior portion of an obliquely longitudinal section through an embryo of the age of Fig. 6, showing cavity in the endoderm.

Figs. 23—27.—Successive transverse sections through an embryo of the age of Fig. 7.

Fig. 28.—Transverse section through the optic ganglion at the age of Fig. 8.

Fig. 29.—Transverse section through the thoracico-abdomen at the age of Fig. 8.

Fig. 30.—Horizontal section through the abdominal papilla, at stage between Fig. 8 and Fig. 9.

Figs. 31—33.—Successive horizontal sections through the abdominal papilla at the age of Fig. 9. The sections are parallel to the line $x\,y$ in Fig. 34, and perpendicular to the plane of the paper.

Fig. 34.—Slightly lateral longitudinal section through Fig. 9.

Fig. 1.

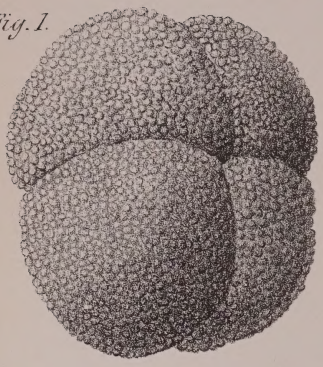


Fig. 5.

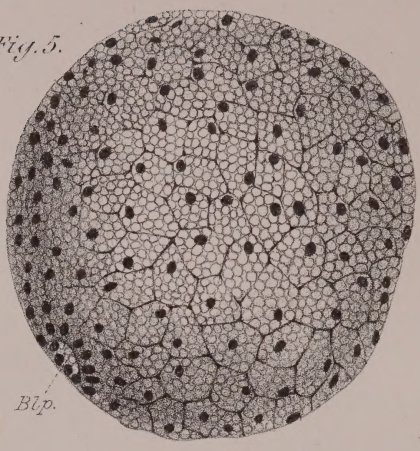


Fig. 5a.

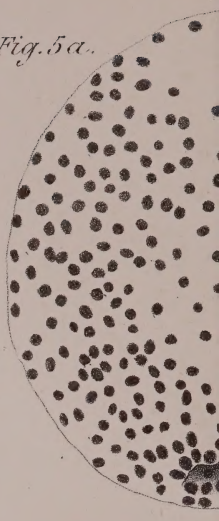


Fig. 2.

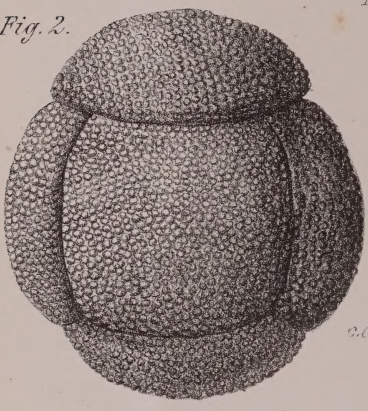
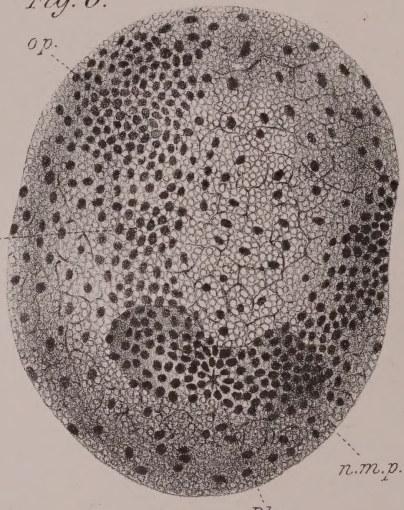


Fig. 6.



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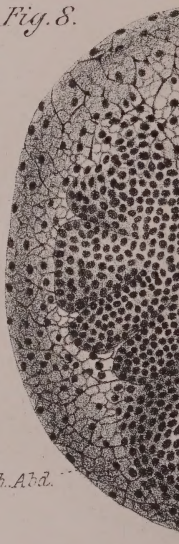


Fig. 3.

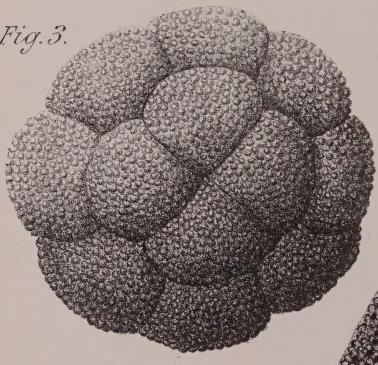
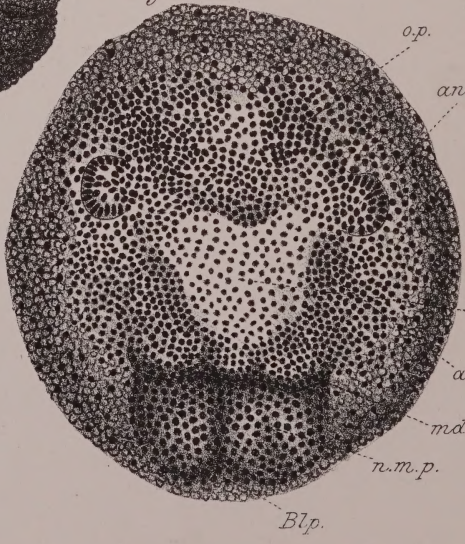


Fig. 7.



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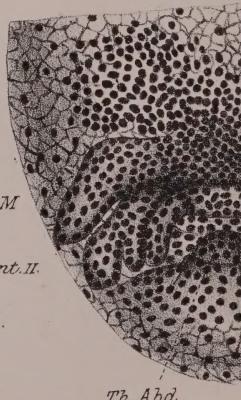

Fig. 4.

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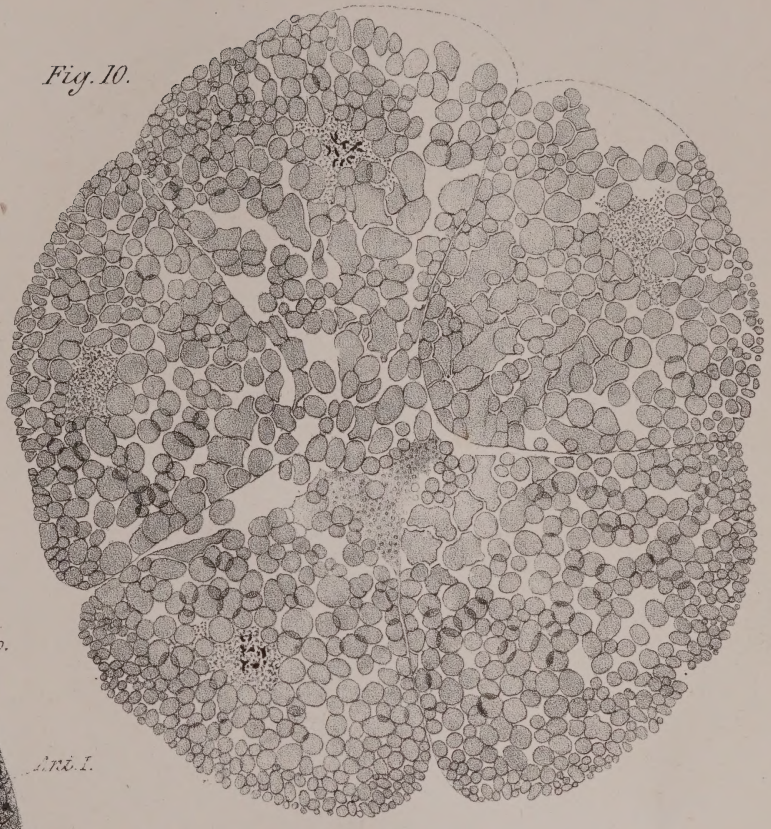
Fig. 10.



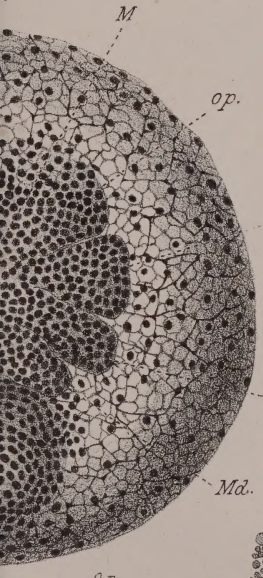
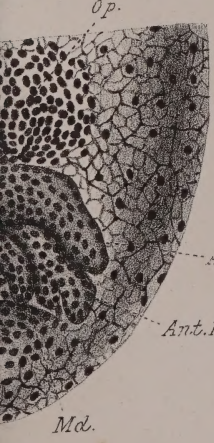
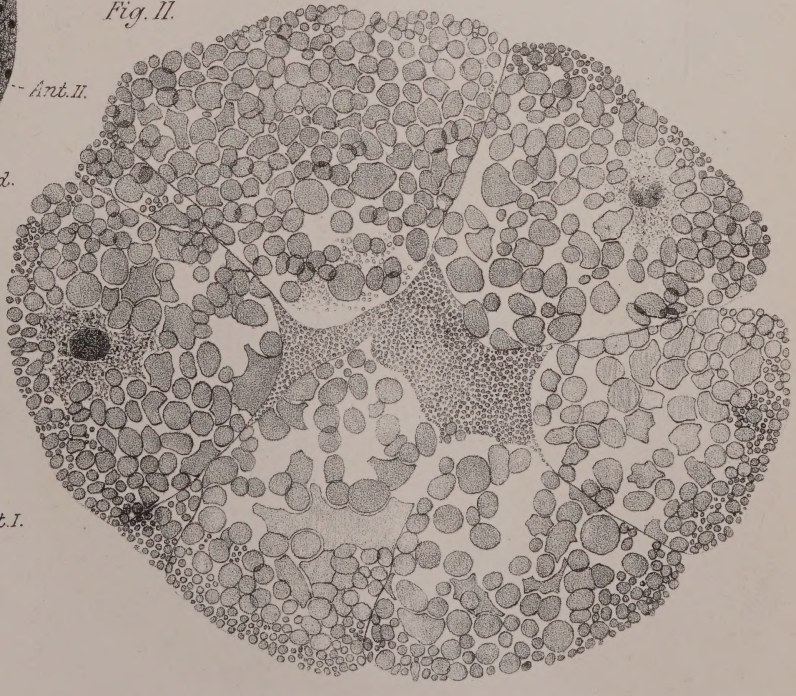


Fig. 11.





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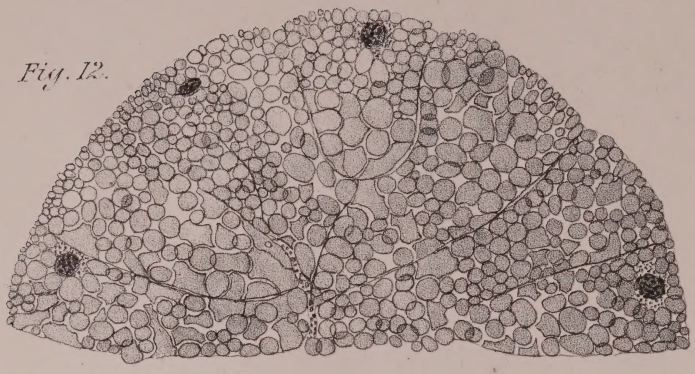

Fig. 12.

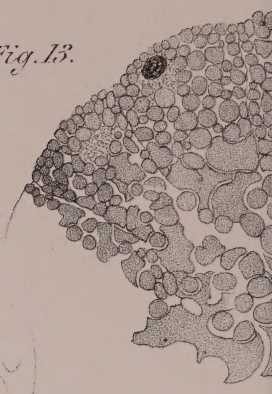

Fig. 13.

Fig. 15.

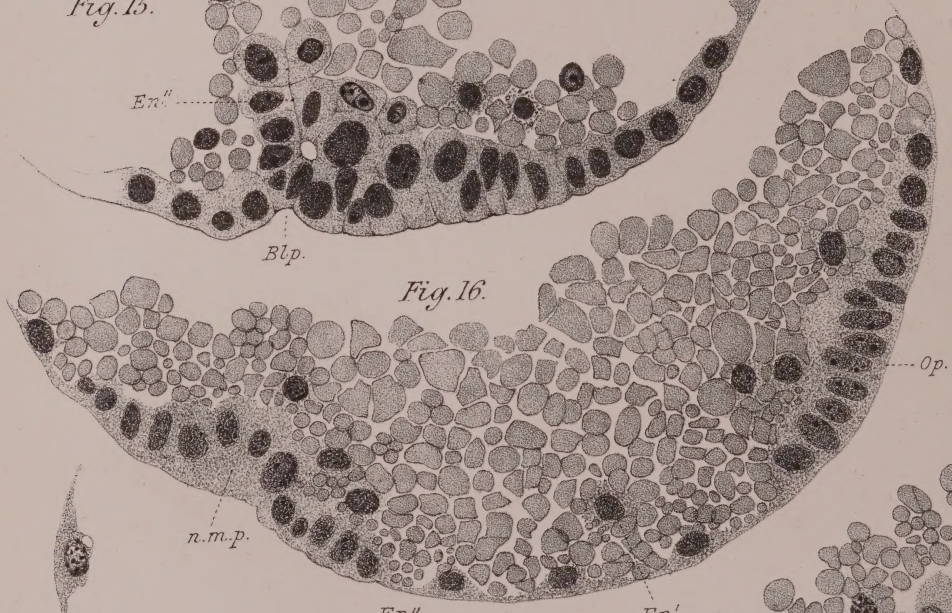

Fig. 16.

Fig. 21.

Fig. 17.

Fig. 22.

Weldon del.

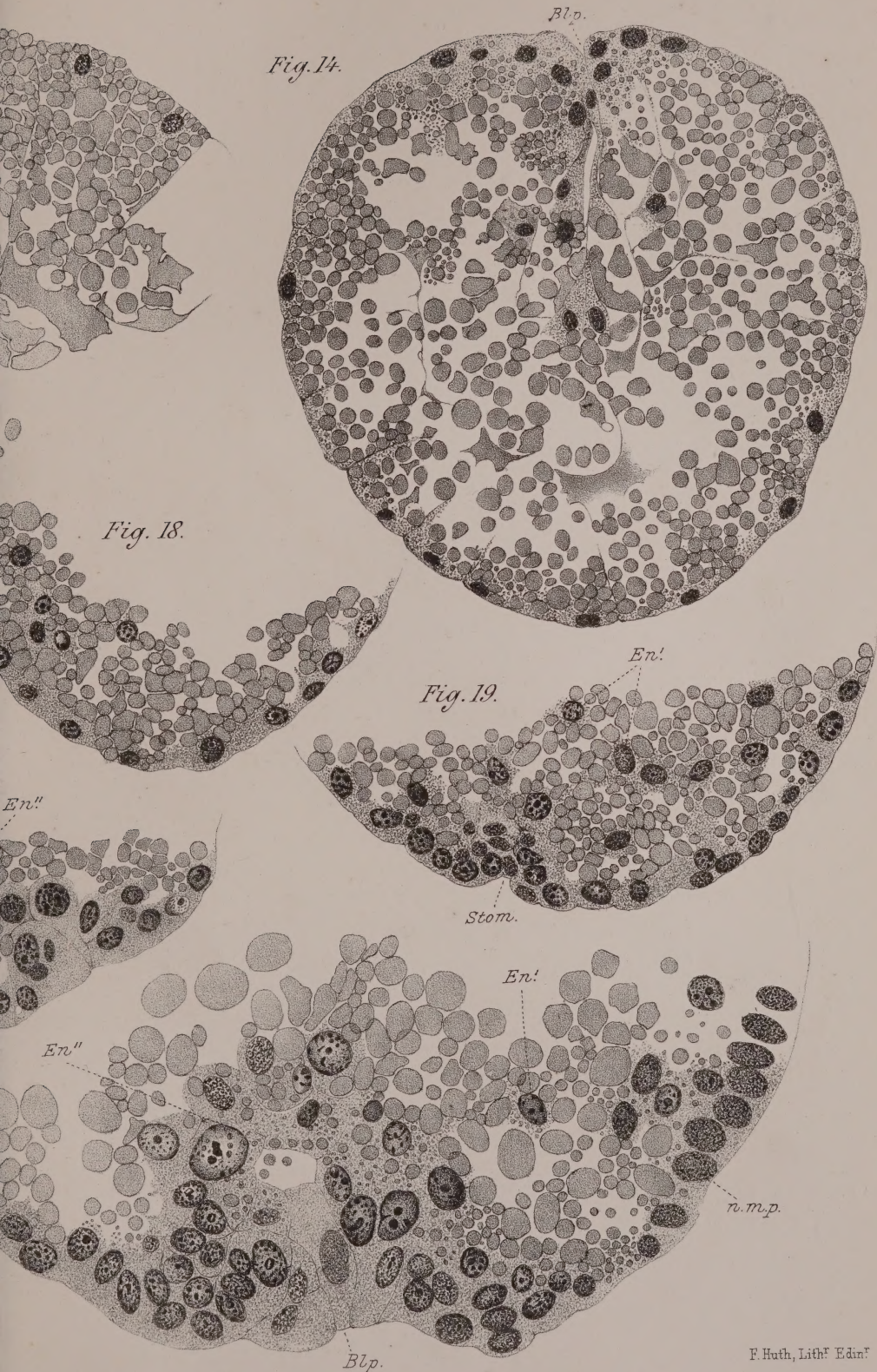

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Fig. 23.
En.I
Ant.I.

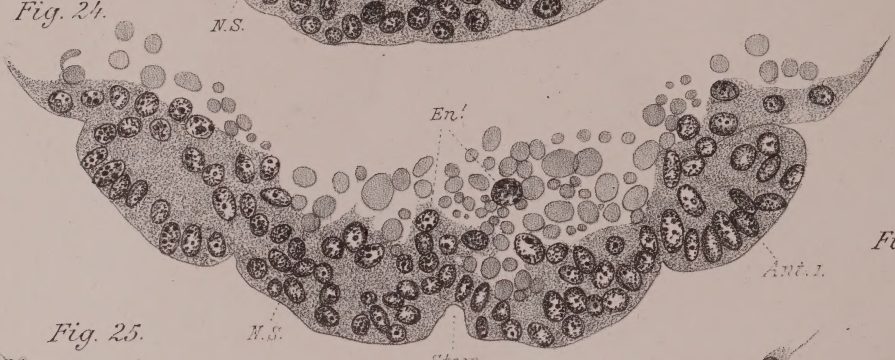
Fig. 24.
N.S.
En.I
Ant.I.

Fig. 25.
N.S.
Stom.
En.I
Ant.II.
N.S.
N.S.
Ant.II.

Fig. 26.
En.I
Md.

Fig. 27.
En.II
n.m.v

Fig. 32.

Weldon del.

Fig. 28.

Fig. 30.

Fig. 31.

Fig. 33.

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